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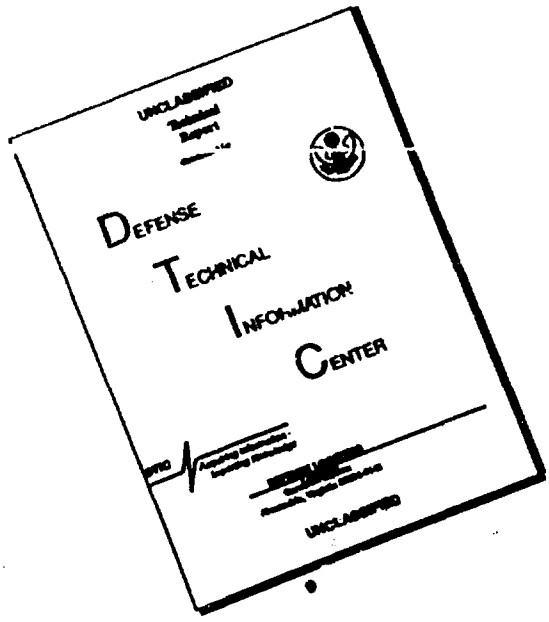

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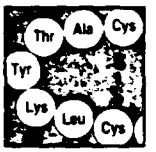
Experimental Hematology

Hematopoietic growth factors and glucocorticoids synergize to mimic the effects of IL-1 on granulocyte differentiation and IL-1 receptor induction on bone marrow cells in vivo



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Abstract. The mechanisms by which interleukin-1 (IL-1) stimulates hematopoiesis are not clear. We have previously shown that in vivo administration of IL-1 indirectly increases IL-1 receptor (IL-1R) expression on both immature and mature bone marrow (BM) cells, partly due to IL-1-induced hematopoietic growth factor (HGF) production. Because IL-1 also stimulates the hypothalamic pituitary-adrenal axis resulting in the production of glucocorticoids (GC), we assessed whether in vivo treatment with HGF and glucocorticoids upregulates IL-1R. Administration of IL-1 to adrenalectomized mice reduces by 53% IL-specific binding on light density bone marrow (LDBM) cells compared to sham-operated mice. The administration of dexamethasone (dex) alone induced only a slight increase in IL-1R expression but synergized with granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage CSF (GM-CSF), IL-3 and IL-6 to upregulate IL-1R expression. Flow cytometry analysis using the RB6-8C5 antibody, which is differentially expressed on myeloid cells, indicated that combined G-CSF and dex treatment acts to promote increased numbers of differentiated myeloid progenitors in the bone marrow. Autoradiographic analysis confirmed that while G-CSF and dex increased IL-1R expression on all myeloid cells, it was particularly pronounced for myelocytes, promyelocytes and metamyelocytes. These results suggest that the ability of IL-1 to enhance granulocyte differentiation in vivo is partly due to its ability to induce a cascade of cytokines and steroids which in turn regulate IL-1 receptor expression.

Key words: IL-1 receptor—CSFs—Glucocorticoids—Myelopoiesis

Introduction. Interleukin-1 (IL-1) is a pleiotropic cytokine affecting the immune, inflammatory, neuroendocrine and hematopoietic systems [1,2]. Much of this diversity is based on the ability of IL-1 to induce production of other biologic mediators such as corticotropin releasing factor, corticosteroids, adrenocorticotropic hormone (ACTH), insulin and hematopoietic growth factors (G-CSF, GM-CSF, IL-6, IL-8, transforming growth factor beta [TGF-ß] and tumor necrosis factor alpha [TNF-α]) from multiple cell types.

The ability of IL-1 to synergize with HGFs to promote the growth and differentiation of primitive progenitor cells in vitro [3-5] suggests that IL-1 also plays an important role in the regulation of hematopoiesis [6]. In vivo administration of IL-1 induces an initial rapid mobilization of neutrophils from the bone marrow, followed by cycling of hematopoietic progenitor cells resulting in the expansion of the granulocytic compartment [7-11]. These effects presumably contribute to the ability of IL-1 to accelerate the recovery of hematopoietic stem cells and blood neutrophils following myelosuppression by chemotherapeutic drugs or exposure to lethal radiation [12-16].

In order to better understand the mechanism of action of IL-1 in vivo, we demonstrated that the IL-1 mediated upregulation of IL-1 receptors on bone marrow cells after in vivo administration of IL-1 occurs by an indirect mechanism [17]. In contrast, several hematopoietic growth factors, such as GM-CSF, G-CSF, IL-3 and IL-6 but not IL-1, act in vitro to upregulate IL-1R on both progenitor cells [18] and mature myeloid cells [18,19]. In vivo administration of HGFs, however, only partially mimic the ability of IL-1 to upregulate IL-1R expression [17]. It is, therefore, likely that other factors also contribute to the in vivo upregulation of IL-1 on hematopoietic progenitor cells. Since the in vivo administration of IL-1 also results in an elevation of corticosteroids in plasma [20] and glucocorticoids enhance in culture the expression of IL-1R on monocytes, B lymphocytes and fibroblasts [21,22], GCs might participate in the upregulation of IL-1R on bone marrow cells observed in response to IL-1. In this study, evidence is presented for a role for GCs as well as HGFs in IL-1R regulation.

Materials and methods

Mice. CD2F1 male mice were purchased from the Animal Genetics and Production Branch, National Cancer Institute, NIH (Frederick, MD). Animals were handled as previously described [23]. Animal care was provided in accordance with the procedures outlined in the *Guide for Care and Use of Laboratory Animals* (NIH Publication #86-23, 1985).

Reagents. Human recombinant IL-1α was supplied by Hoffmann-La Roche (Nutley, NJ). Human recombinant G-CSF was supplied by Amgen Corp. (Thousand Oaks, CA). Human recombinant IL-6 (5×10^6 U/mL) in pyrogen-free solution was kindly provided by Dr. Menachem Rubinstein (Interpharm

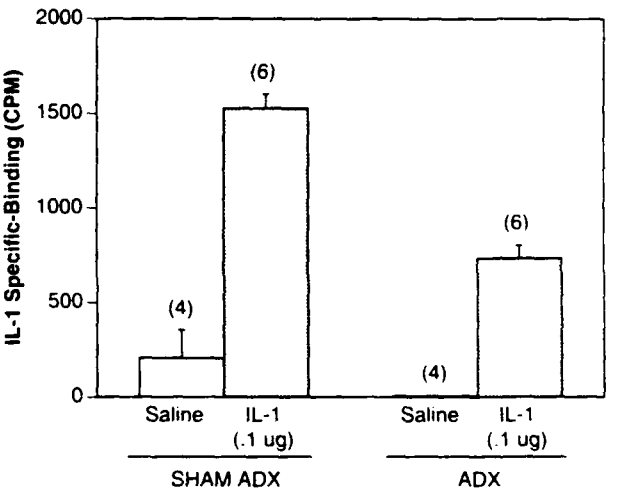

Fig. 1. Comparative effect of in vivo IL-1 on IL-1R expression on BM cells from adrenalectomized (removal of adrenal gland) mice vs. sham-adrenalectomized (similar surgical procedure without removal of the adrenal gland) mice. Mice were adrenalectomized 14 days before injection of 100 ng IL-1. Sixteen to 18 hours after IL-1 administration, BM cells were harvested and tested for the expression of IL-1R as described in Materials and methods. Each bar represents the mean ± SE of 2 experiments from which the level of background binding (280 ± 41) was subtracted. The numbers in parentheses represent the number of mice receiving each treatment.

Laboratory, Ness-Ziona, Israel). Murine IFN-γ was kindly provided by Genentech (San Francisco, CA). Murine recombinant IL-3 and GM-CSF were generously provided by Dr. Steven Gillis (Immunex Corp., Seattle, WA). Human recombinant TGF-ß was generously provided by Dr. Tony Purchio (Bristol-Myers/Squibb Pharmaceuticals, Seattle, WA). Dexamethasone sodium phosphate was purchased from LymphoMed Inc. (Rosemont, IL).

In vivo procedures. Cytokines and dexamethasone were diluted in pyrogen-free saline on day of injection. Predetermined optimal doses of cytokines [17,23,24] were given intraperitoneally (i.p.) at the same time as dex administered at 50 µg/mouse or at the dose indicated in the text. Bone marrow cells were tested for the expression of IL-1R 16 to 18 hours after treatment. Adrenalectomy was performed under anesthesia 14 days before the experiment. Adrenalectomized mice were given 1.0% (wt/vol) NaCl in drinking water.

Measurement of CSF activity in serum. CSF titer in serum was measured as follows. Briefly, mice were bled 2 to 3 hours after IL-1 injection and serum was collected by centrifugation after clot formation. CSF activity was determined using bone marrow colony assay for CSF activity as previously described [20]. Briefly, BM cells were suspended in 1.0 mL IMDM, 10% FCS, in 0.3% Seaplaque agarose (Rockland, ME) in the presence or absence of serum (serial 2-fold dilutions). The cells were plated in 35 mm Lux petri dishes (Miles Laboratories Inc., Naperville, IL) and incubated at 37°C in 5.0% CO_2 and scored for colonies (>50 cells) growth after 7 days of incubation. CSF activity was expressed as colony-forming units per millimeter, based on the colony count at 50% of maximum response (Ed50).

Preparation of bone marrow cells. Murine bone marrow cells were aspirated from femurs and low-density mononuclear cells were isolated by separation on Lymphocyte Separation Medium (Organon Teknika Corp., Durham, NC).

Flow cytometry analysis of bone marrow cells. BM cells from saline-, dex- and/or cytokine-treated mice were labeled with either monoclonal antibody (MAB) RB6-8C5, Thy-1, L3T4, F480 or control IgG in an indirect immunofluorescence assay. Briefly, 10^6 BM cells were resuspended in RPMI with 10% fetal calf serum and incubated for 30 minutes at 4°C with 1.0 µg of the appropriate MAB. The cells were washed and then incubated with fluorescein-labeled goat antirat antibody for 30 minutes at 4°C. The cells were then washed 2 times with PBS alone, fixed with 1.0% paraformaldehyde in PBS and analyzed using Coulter Profile II.

Preparation of iodinated IL-1. Human rIL-1α was labeled with ^{125}I using chloramine-T reagent as described previously [25]. The radiolabeled IL-1α had a specific activity that ranged from 1 to $3x10^{15}$ cpm/mmol. There was no significant loss of biological activity of radiolabeled IL-1α as determined by the thymocyte comitogenic activity assay.

Receptor binding assay. Fractionated bone marrow cell suspensions were washed once with cold medium and cell pellets were treated for 1 minute on ice with 50 mm glycine-HCl (pH 3.0) to remove potentially bound cytokines. Subsequently, the cells were washed twice with binding medium (RPMI 1.0% BSA supplemented with 0.1% sodium azide and 10 mm Hepes) and incubated at 4°C with 500 pm ^{125}I-labeled IL-1α in a final volume of 0.2 mL. After 1 to 2 hours of incubation, cell-bound radioactivity was separated from unbound ^{125}I-IL-1α by centrifugation of the sample through a mixture of 1.5:1 (vol/vol) dibutyl phthalate/bis(2-ethylhexyl)-phthalate (Eastman Kodak Co., Rochester, NY). Nonspecific binding was determined by incubating bone marrow cells with labeled IL-1α in the presence of 50-fold excess of unlabeled ligand.

Autoradiography. LDBM cells from mice treated 16 to 18 hours with IL-1, G-CSF and/or dex were prepared as described for receptor binding assay and incubated at 4°C with 1.0 nm ^{125}I-IL-1α. After 1 hour of incubation, cell-bound radioactivity was separated from unbound IL-1 by centrifugation of the sample through a layer of cold FBS. The autoradiography was performed using a modification of a previously described technique [26]. Briefly, $2x10^5$ cells were centrifuged onto microscope slides coated with 0.5% gelatin, fixed in methanol for 10 minutes, coated with Kodak NTB2 photographic emulsion and exposed at 4°C for 4 weeks. Slides were developed with Kodak D-19 developer, fixed with Kodak fixer, stained with Jenner-Giemsa. The number of grains was determined for over 50 cells per slide for 2 slides.

Results

Endogenous corticosteroid production is involved in the upregula-

Table 1. IL-1-stimulated CSF production in adrenalectomized and sham-adrenalectomized mice

Mice[a]	IL-1 injection (ug/mice)[b]	CSF titer (U/mL)[c]
Sham-ADX	None	< 20
Sham-ADX	0.1	590
Sham-ADX	1.0	705
ADX	None	< 20
ADX	0.1	750
ADX	1.0	853

[a]Mice were either adrenalectomized or sham-adrenalectomized as described [23].
[b]Mice were bled 2 to 3 hours after IL-1 injection as described in Materials and methods.
[c]Serum was assayed for CSF activity as described in Materials and methods. The data represent CSF titers of pooled serum from 3 mice.

tion of IL-1R. To examine the role of endogenous corticosteroids in the upregulation of IL-1R on bone marrow cells after IL-1 injection, mice were adrenalectomized. The highest dose of IL-1 (100 ng/mouse) that adrenalectomized mice could tolerate was given i.p. 14 days after surgery. As already demonstrated in normal animals [17], the administration of 100 ng of IL-1 to sham-adrenalectomized mice resulted in a 15-fold increase in IL-1-specific binding compared to saline-treated sham-adrenalectomized mice at 16 hours after injection of IL-1 (Fig. 1). This time was chosen based on our previous studies showing maximal IL-1R expression at 16 to 18 hours [17]. The specific binding of IL-1 on BM cells from IL-1-treated adrenalectomized mice was reduced by 53% compared with IL-1-treated sham-adrenalectomized mice. No specific IL-1 binding was detectable on bone marrow cells from adrenalectomized mice. These data suggest that endogenous corticosteroids participate in the constitutive and IL-1-induced expression of IL-1R.

Since it has been established that IL-1-induces HGF production [2,23], we studied whether adrenalectomy influences IL-stimulated HGF production. For this, adrenalectomized and control (sham-adrenalectomized) mice were bled 2 to 3 hours after IL-1 injection. IL-1 induced similar levels of CSF production in all mice tested (Table 1), indicating that adrenalectomy does not impair IL-stimulated HGF production. We next examined the ability of exogenous addition of dex to synergize with IL-1 in the regulation of IL-1R expression. The injection of optimal concentrations of dex (50 μg) [27] in the presence of optimal amounts of IL-1 (1.0 μg) [17] did not increase IL-1R expression (Fig. 2). At concentrations of 0.1 and 0.3 μg of IL-1 per mouse, however, dex increased IL-1R expression 2-fold The magnitude of IL-1 upregulation in these cases, however, did not equal the magnitude seen with optimal IL-1 concentrations.

Dexamethasone synergizes with HGFs in the upregulation of IL-1R. Since we have recently determined that increased IL-1R expression after IL-1 administration to mice was mediated, in part, through endogenous HGF production [17], we examined the effect of dexamethasone in combination with HGFs on IL-1R expression on BM cells. Mice were injected with equivalent doses of hematopoietic growth factors such as G-CSF, GM-CSF, IL-3 and IL-6 as well as TGF-ß, a negative regulator of hematopoiesis. Among the cytokines tested, G-CSF, GM-CSF, IL-3 or IL-6 treatment in combination with dex (50 μg) results in a synergistic effect on IL-1R expression on bone marrow cells (Fig. 3). At equivalent doses, rG-CSF was slightly more potent than GM-CSF, followed by IL-6 and IL-3 (Fig. 3). G-CSF plus dex and GM-CSF plus dex were equal to or better than IL-1. TGF-ß alone did not increase IL-1R expression and did not synergize with dexamethasone.

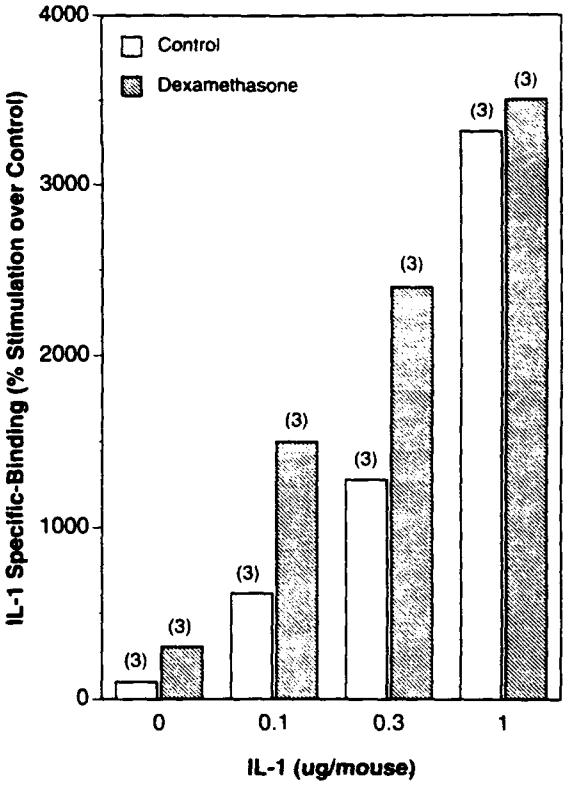

Fig. 2. Dose-dependent interaction between dex and IL-1 administration for increase in IL-1R expression
Mice were injected i.p. with either saline, the indicated doses of IL-1 and maximal dose (50 μg) of dex. Sixteen to 18 hours after treatment radioreceptor assays for the expression of IL-1R on BM cells were done as described in Materials and methods. The data represent the mean ± SE of duplicate determinations using pooled cells from 3 animals. Background was 259 ± 34, which was subtracted from the data shown.

Using G-CSF, the dose-dependence of the synergistic interaction between dex and HGFs was studied. The administration of a previously determined [17] optimal dose of G-CSF significantly increased the expression of IL-1R on BM cells (4.3-fold) (Fig. 4). The administration of dex alone induced a 2-fold increase in IL-1R expression but synergized with G-CSF to upregulate IL-1R in vivo in a dose-dependent fashion (Fig. 4). In all experiments performed (n=7), the administration of G-CSF (5.0 μg) and dex (50 μg) upregulated IL-1R expression to a greater extent than seen with an optimal dose (1.0 μg) of IL-1 alone (Fig. 4). This synergy with G-CSF in increasing IL-1R expression was dose-dependent on dex (Fig. 4).

To determine if G-CSF and dex can act on isolated hematopoietic cells in vitro, normal BM cells were incubated in vitro with G-CSF and/or different concentrations of dex. G-

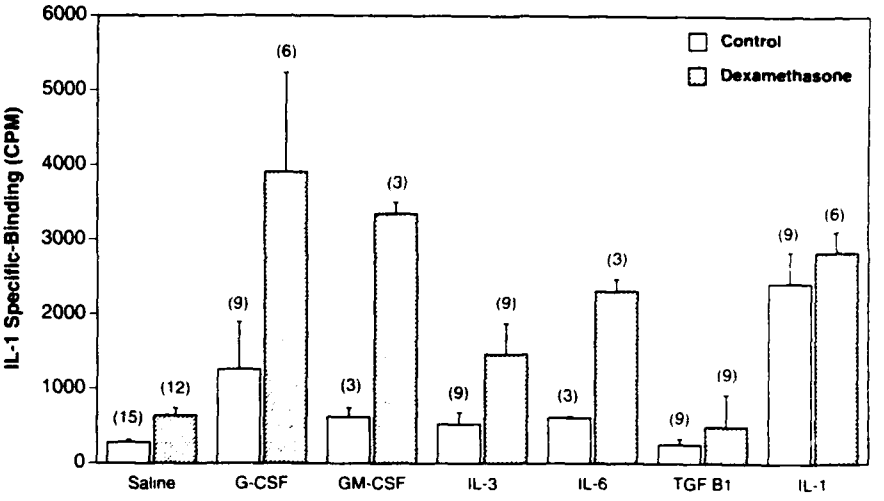

Fig. 3. Hematopoietic growth factors synergize with dexamethasone to upregulate IL-1R expression
Mice were injected i.p. with either saline, IL-1 (1.0 μg), G-CSF (5.0 μg), GM-CSF (5.0 μg), IL-3 (5.0 μg), IL-6 (5.0 μg), TGF-ß (5.0 μg) and/or dex (50 μg). Sixteen to 18 hours after treatment, BM cells were harvested and tested for the expression of IL-1R using radioreceptor assay. The data represent the mean ± SE of determinations using 3 to 15 mice as indicated in parentheses, 2 to 4 experiments for each cytokine. The level of background binding was 315 ± 52, which was subtracted from the data shown here.

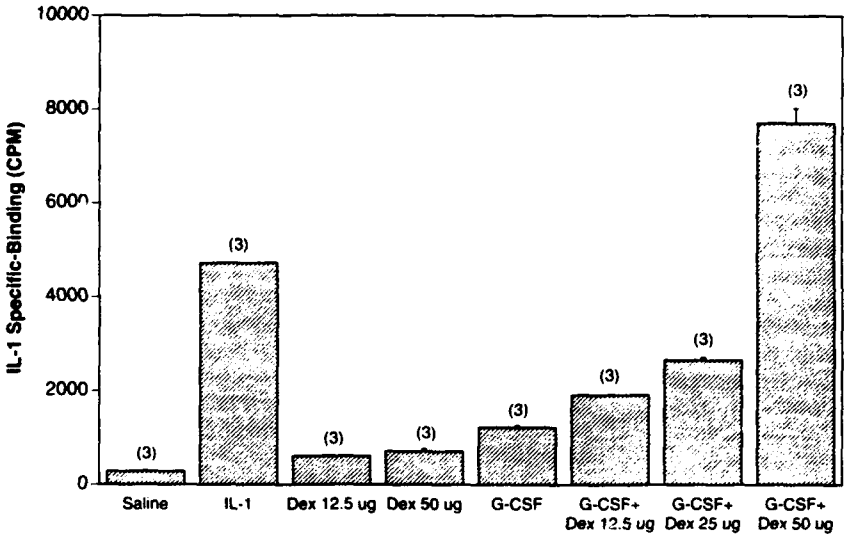

Fig. 4. Dose-dependent synergistic interaction between dexamethasone and G-CSF administration for the increase in IL-1R expression
Mice were injected i.p. with either saline, maximal dose of IL-1 (1.0 μg), maximal dose of G-CSF (5.0 μg) and/or increasing doses of dex. Sixteen to 18 hours after the treatments, radioreceptor assay for the expression of IL-1R on BM cells was performed as described in Materials and methods. The data represent the mean ± SE of duplicate determinations of at least 2 experiments using pooled cells from 3 animals. The level of background binding was 259 ± 34, which was subtracted from the data shown here.

CSF and dex also synergize in vitro to increase IL-1-specific binding (Table 2), whereas IL-1 by itself had no effect.

Distribution of IL-1R on bone marrow cells from G-CSF- and dex-treated mice. We next evaluated whether the in vivo administration of G-CSF and GC increased expression of IL-1R on a specific population or subpopulation of BM cells. Mice received a single injection of saline or a combination of G-CSF and dex. IL-1 binding to BM cells was determined by autoradiography, 16 to 18 hours after injection. Autoradiographic analysis of cells from saline-treated animals showed that most of the labeled cells belonged to the granulocytic series (Table 3, Fig. 5). Seven percent of the undifferentiated blast/early cells were labeled with 8 grains per cell. After treatment with G-CSF and dex, these cells were 19% positive with 28 grains per cell. Promyelocytes and myelocytes (43% positive with 19 grains per cell) were the most heavily labeled cells after G-CSF and dex treatment (87% positive with 57 grains per cell). Thirteen and 16% of eosinophilic and monocytic cells exhibited a similar pattern of labeling with 7 to 8 specific grain per cell. More monocytic cells were labeled after G-CSF and dex treatment (31%) with a small increase in the number of grains per cell (8 to 17). This treatment had no effect on eosinophilic cells. No specific IL-1 labeling was observed on erythroid cells. These results clearly demonstrate that G-CSF and dex treatment results in an increase in IL-1R expression along the myelocytic series and is particularly pronounced for myelocytes followed by metamyelocytes and segmented neutrophils.

G-CSF and dexamethasone promote granulopoiesis. Because the administration of IL-1 induces an initial rapid mobilization of neutrophils from the bone marrow followed by increased cycling of hematopoietic progenitor cells, resulting in the expansion of granulocytes in the marrow [7-11] we examined whether the combination of G-CSF and dex also promoted an expansion of myeloid cells. Bone marrow cells were analyzed by fluorescence-activated cell sorting accordingly to the differential expression of RB6-8C5 antigen on myeloid cells [28].

Table 2. In vitro interaction between G-CSF and dexamethasone in the upregulation of IL-1R on bone marrow cells

Factor added[a]	IL-1-specific binding (CPM)[b]
None	798 ± 56
DEX 10^{-9}M	1341 ±30
G-CSF	4195 ± 273
G-CSF + DEX 10^{-10}M	5985 ± 409
G-CSF + DEX 10^{-9}M	8685 ± 22
IL-1	747 ± 220

[a]LDBM cells obtained from normal mice by Ficoll separation were incubated 24 hours in the presence or absence of the 20 ng/mL of IL-1 or G-CSF and the indicated concentrations of dex.
[b]Bone marrow cells were treated for IL-1 binding as described in Materials and methods. The data represent the mean ± SEM of duplicate determination of a representative experiment of 2 experiments. The level of background binding was 402 ± 168 cpm which was subtracted from the total cpm to give the data shown here.

Table 3. Distribution of IL-1R on BM cells from G-CSF plus dexamethasone–treated mice

	Specific IL-1 binding			
	% labeled		mean grain count	
Cell type	saline	G-CSF + Dex	saline	G-CSF + Dex
Blasts/ early cells	7	19	8	28
Promyelocytes/ myelocytes	43	87	19	57
Metamyelocytes	29	72	11	30
Later neutrophils	23	39	7	23
Eosinophils	13	13	7	8
Monocyte	16	31	8	17
Nucleated erythroid	0	0	0	0

Data represent background-subtracted grain count over more than 20 cells of each type. The level of background binding of >5 grains was subtracted from the total grain count to give the data shown here.

The RB6-8C5^{hi} cells are enriched for the end stage (segmented) neutrophils (>75%) while the RB6-8C5^{lo} cells are enriched for myeloblasts, promyelocytes and myelocytes (>80%). The RB6-8C5^{lo} population represents 19 to 24% of total bone marrow and contains 50% of CFU-GM progenitors [28]. As previously demonstrated [28], the administration of IL-1 to mice results in a 206% increase in the RB6-8C5^{lo} population and a concomitant 41% loss of RB6-8C5^{hi} (Fig. 6, Table 4). Treatment of mice with G-CSF and dex induced a 406% increase in the RB6-8C5^{lo} immature myeloid population and no reduction in the RB6-8C5^{hi} population. In comparison, dex and G-CSF promoted a 206% and 218% increase in RB6-8C5^{lo} population, respectively, and a 137% increase and a 45% reduction in the RB68C5^{hi} population, respectively.

Discussion

We have previously demonstrated that injection of mice with IL-1 results in considerable upregulation of type II IL-1R on myeloid-enriched progenitors [17]. By the administration of antibody against type I IL-1R not present on bone marrow progenitors, we have clearly demonstrated that this upregulation occurs through an indirect mechanism. Administration of IL-1 in vivo stimulates the hypothalamic-pituitary-adrenal axis, resulting in the production of GC [20] as well as HGF production by type 1 IL-1R expressing accessory cells [29-32]. Both GC and HGF are elevated rapidly, with maximal levels reached within 2 hours after IL-1 administration [20,29]. GC have been previously shown to upregulate IL-1R on human monocytes and B cells in vitro [21,22].

In this report, evidence is presented that IL-1-induced endogenous GC synergize with HGF to mediate IL-1 responsiveness on bone marrow cells. The injection of IL-1 to adrenalectomized mice reduced the upregulation of IL-1R by 53% compared with sham-adrenalectomized mice. This effect, which is associated with diminished GC production, is not due to impaired IL-1-induced HGF production in adrenalectomized animals (Table 1). The concomitant administration of GC and HGF such as G-CSF, GM-CSF, IL-3 and IL-6, to normal mice synergistically increased functional IL-1R on bone marrow cells. In addition, this synergy between GC and HGF was also seen in vitro. The observations concerning glucocorticoid modulation of IL-1R in vivo using adrenalectomized mice and the synergy between G-CSF and dex are in agreement with a recent observation from Shieh et al. [19]. While the data indicate a role for GC in IL-1 receptor regulation, other mechanisms cannot be excluded.

In addition, we found that GM-CSF and G-CSF were equally potent in synergizing with dex while IL-6 and IL-3 were approximately 50% as potent. Unlike IL-1, HGF and GCs can stimulate IL-1R expression on hematopoietic cells in vitro [33, 34]. Whether this is a direct effect must await the results of technically difficult binding and antibody-blocking assays on single cells.

Because administration of IL-1 rapidly induces an initial mobilization of neutrophils from the bone marrow followed by increased cycling of hematopoietic progenitor cells, resulting in the expansion of granulocytic compartment in the bone marrow [20,23], we examined whether G-CSF and dex have the same effect. Bone marrow cells were analyzed by fluorescence-activated cell sorting according to the differential expression of RB6-8C5 antigen on myeloid cells [28]. We had previously shown that RB68C5^{hi} cells are enriched for the end stage (segmented) neutrophils (>75%) while the RB6-8C5^{lo} cells are enriched for myeloblasts, promyelocytes and myelocytes (>80%). While the administration of IL-1 to mice results in an 206% increase in the RB6-8C5^{lo} population and a concomitant 41% loss of RB6-8C5^{hi}, treatment of mice with G-CSF and dex induced a 406% increase in the RB6-8C5^{lo} immature myeloid population and no reduction in the RB6-8C5^{hi} population. In addition, autoradiographic analysis of IL-1 binding on bone marrow cells after G-CSF and dex treatment showed that while cells in all stages of granulocytic development had increased IL-1 binding, the most dramatic increase in terms of number of cells positive and grains per cell were the myelocytes, promyelocytes and metamyelocytes.

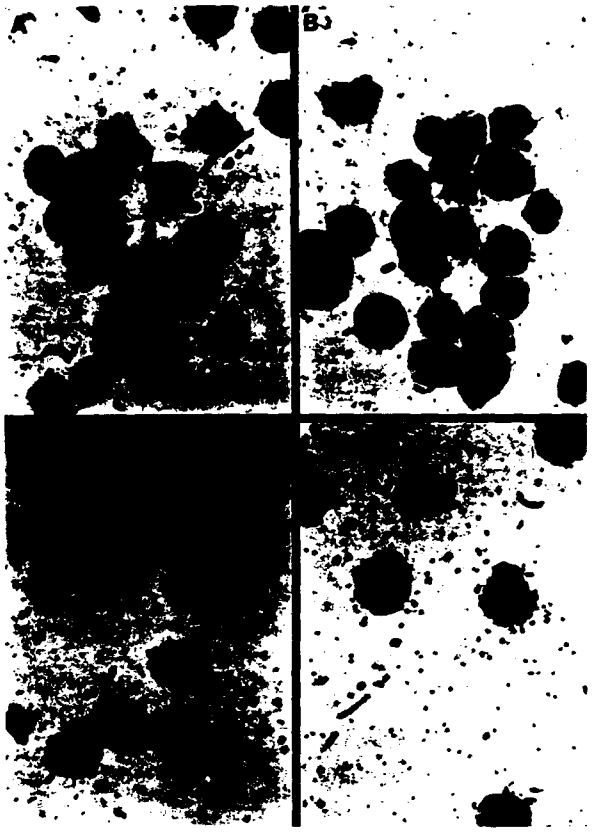

Fig. 5. Autoradiography of ^{125}I-IL-1-labeled LDBM cells from control and G-CSF plus dex-treated mice
Mice received a single i.p. injection of G-CSF (5.0 μg) and dex (50 μg). After 16 hours, bone marrow cells were harvested and radioautography of BMC labeled with ^{125}I-IL-1. Emulsion films were developed after 4 weeks. Panels **A, C** and **D** without cold IL-1; panel **B** with an excess of cold IL-1.

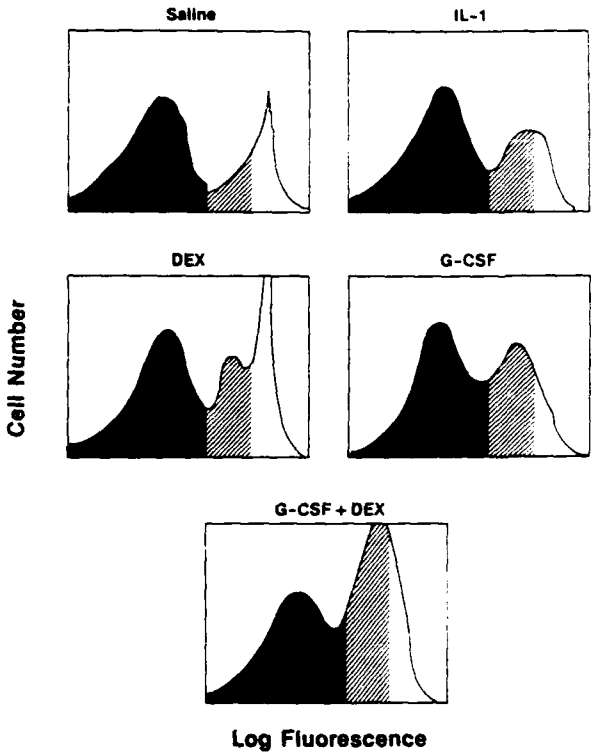

Fig. 6. Differential expression of RB6-8C5 antigen on BM cells from mice treated with G-CSF and/or dexamethasone
Mice were injected i.p. with either saline, IL-1 (1.0 μg), G-CSF (5.0 μg) and/or dex (50 μg). Sixteen to 18 hours after treatment, BM cells were labeled in indirect immunofluorescence assay by using the MAB RB6-8C5 as outlined in Materials and methods. The cells were gated according to fluorescent intensity into 8C5^{neg} (solid region) showing fluorescence between channels 0 and 60, 8C5^{lo} (hatched region) between 60 and 175 and 8C5^{hi} (open region) between 175 and 250. The background staining of the isotype-matched control antibody was less than 3.5% for each treatment.

Whether the increased IL-1R binding leads to increased IL-1 responsiveness is being studied. Thus, it is shown that G-CSF and dex in combination in vivo mimic the effects of IL-1 in granulocyte differentiation.

In addition, we have previously shown that an antibody against type I IL-1R not expressed on neutrophils blocked most of the initial mobilization of neutrophils together with HGF production by type I–expressing stromal cells [17,23]. This confirms that chemotactic response of neutrophils to IL-1 is indirect, probably mediated through IL-1-induced potent chemotactic cytokines such as IL-8 [35,36]. In this report, we show that G-CSF alone can mimic the extent of the initial mobilization of bone marrow neutrophils due to IL-1 (Fig 6, Table 4). Unlike IL-1, G-CSF is directly chemotactic for neutrophils in vitro [37]. It is therefore likely that G-CSF participates with other cytokines induced by IL-1 in the mobilization of neutrophils observed after IL-1 administration.

In general, the amplitude of the response to IL-1 correlates with cell surface receptor expression. For example, positive regulators such as PDGF increased the number of IL-1R together with the capacity of the cell to respond to IL-1 [38]. In addition, treatment of hematopoietic progenitor cells with negative regulators such as TGF-ß blocked the ability of IL-1 to promote high proliferative potential (HPP) colony formation as well as greatly reduced the expression of IL-1R expression [39]. Clinically, increased IL-1R expression has been noted in sepsis, organ failure and acute disseminated inflammation [40]. In this report, concomitant injection of HGF and GC increased IL-1R expression on myeloid cells in the bone marrow with the most increase seen on the myelocyte and promyelocytes followed by metamyelocytes and segmented neutrophils. Such an increase in IL-1R on a premitotic population would serve to promote cellular differentiation and/or cell division, resulting in an amplification of granulocyte differentiation. Therefore, the ability of IL-1 to enhance granulopoiesis in normal [7-11] as well as in myelosuppressed [12-16] mice may be partly due to the unique ability of IL-1 to induce a complex cascade of cytokines and steroids, which can then act to regulate IL-1 receptor expression.

In conclusion, these results provide new insights into the mechanism of IL-1 restorative effects in the marrow. IL-1 stimulates production of HGF and GC which in turn upregulate the expression of IL-1R and render the cells more responsive to IL-1. This accounts for the initial burst of granu-

Table 4. Flow cytometric analysis of BM cells from dex- and G-CSF-treated mice using RB6-SC5 antibody

Treatments[a]	Populations[b] RB6-8C5^{lo}	RB6-8C5^{hi}
Saline	7.7 ± 2.4 (100)	15.8 ± 1.1 (100)
IL-1	16.3 ± 4.6 (210)	9.8 ± 1.3 (62)
Dex	15.4 ± 5.6 (200)	23.3 ± 0.9 (147)
G-CSF	23.6 ± 2.2 (306)	11.8 ± 1.6 (74)
G-CSF + Dex	31.3 ± 7.4 (406)	18.0 ± 601 (114)

[a]Mice were injected i.p. with either saline, maximal doses of IL-1 (1 ug), G-CSF (5 ug) and/or dex (50 ug). Sixteen to 18 hours after treatment, 10^6 BM cells were labeled in an indirect immunofluorescence assay by using the MAB RB6-8C5 as outlined in Materials and methods. Background staining of the isotype-matched control antibody was >3.5% for each treatment. Data represent mean ± SEM for 2 experiments using pooled cells from 3 mice.
[b]The numbers in parentheses represent the percent of stimulation and inhibition of controls (saline-treated). Using an established protocol (28), 8C5^{neg} showed fluorescence between channels 0 and 60, 8C5^{lo} between 60 and 175 and 8C5^{hi} between 175 and 250.

lopoiesis seen after administration of even nanogram amounts of IL-1.

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